Penicillin hypersensitivity – is milk a significant hazard?: a review[1]

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Introduction

It has been recognized for many years that hidden sources of penicillin might represent a hazard to penicillin-allergic patients. Coleman & Siegel (1955) were the first to draw attention to this possibility by reporting a case in which penicillin contaminating a sterilized syringe was implicated in the development of anaphylactic symptoms in a patient receiving a testosterone injection. The patient had previously developed generalized allergic reactions after oral therapy with penicillin and also following the use of penicillin skin tests. Bierlein (1956) and Siegel (1959) drew attention to other hidden sources of penicillin, including ingestion of penicillin in vaccines and in contaminated milk.

The purpose of this review is to assess the significance of low levels of penicillins in milk in terms of risk to penicillin-allergic patients and of sensitization *de novo*. Three basic questions are addressed. (1) Does what is known of the fundamental processes of penicillin allergy, in terms of immunochemical pathways and penicillin-derived allergens, provide a mechanistic framework within which a role can be established for penicillin in milk? (2) Is there evidence that low levels of penicillin or penicillin-related substances can act as allergens by the oral route? (3) What is the clinical evidence of risk to individuals or of public health hazard?

Basic mechanisms of penicillin allergy

Penicillin allergy resulting from the therapeutic administration of penicillins is well documented. Table 1 summarizes the main characteristics of penicillin-induced allergic reactions on the basis of the underlying mechanisms. IgE-antibody-mediated reactions are the most significant. The mechanism by which these reactions can lead to acute clinical episodes, most commonly urticarial lesions but, in rare instances, life-threatening anaphylactic reactions, is established. It involves the release of chemical mediators of inflammation from the mast cell, a cell of the granulocyte series, which occupies a pivotal role in these acute, immediate hypersensitivity reactions. Other clinical syndromes may also be relevant to the issue of penicillin allergy resulting from ingestion of milk containing penicillin residues. In particular, it is important to consider the possibility that immune complexes could be formed and initiate both cutaneous reactions and other symptoms reminiscent of serum sickness. Contact allergic reactions are unlikely to be of significance in this context but may arise as a consequence of handling penicillin preparations for intramammary use.

It is clear that not only are the penicillins capable of initiating immunological reactions but also that there is marked heterogeneity in the immune response to penicillins and consequently in clinical responses also (Levine 1966). The immunochemical pathways involved in the formation of penicillin-derived antigens are well documented and they focus essentially on the concept that the ability of low molecular weight chemical compounds to stimulate a specific immune response is a direct function of reactivity with protein amino groups or other nucleophiles. Thus, the hapten theory of drug immunogenicity states that covalent reaction between the drug or derivative of it and macromolecule is required before an immunological response can be generated (reviewed in Dewdney 1979). The penicillins

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Table 1. Immunological mechanisms involved in penicillin allergy (based on Coombs & Gell 1975)

Type	Immunological mechanism	Manifestation induced by penicillin
I	Release of vasoactive amines from mast cells following interaction between IgE antibody and allergen on surface	Urticaria, angio-oedema, anaphylactic reactions, rapid in onset
II	Interaction of IgM or IgG antibody with penicilloylated erythrocytes with uptake of complement	Haemolytic anaemia
III	Inflammatory reactions due to deposition of antigen-antibody complexes in vascular system and skin	Serum sickness syndrome. Drug-induced fever. Possibly erythematous rashes
IV	Generation of lymphokines or cytotoxicity by T lymphocytes in absence of free antibody	Contact dermatitis

are capable of this protein reactivity. Figure 1 shows that reaction can occur at physiological pH between the β-lactam carbonyl group of the penicillin and protein amino groups to form the penicilloyl determinant. The major population of antibodies generated as a result of penicillin administration in man and animals is directed against this penicilloyl determinant, underlining its importance as a potential allergen. The same determinant can also be formed by reaction of penicillenic acid, a breakdown product of penicillin, with protein amino groups, as shown in Figure 1. It is of interest that penicilloic acid, the major degradation product of penicillin, does not have the ability to react covalently with protein amino groups although there is some evidence of reaction with thiol groups of proteins (Figure 2). It has been claimed that penicilloic acid can act as a minor determinant in penicillin allergy but the precise mechanism has not been clarified. This point will be referred to later in discussion of the use of penicillinase in the prophylaxis and treatment of penicillin-allergic patients.

Thus, mechanisms can be put forward to explain the ability of penicillins to stimulate immune responses. It is worth noting that these immunochemical pathways have been established primarily using benzylpenicillin as a model. It may not be appropriate to extrapolate either qualitatively or quantitatively to the range of semi-synthetic penicillins now available. This is also true of the cephalosporins. These too are β-lactam-containing antibiotics and share with the penicillins protein reactivity through the β-lactam carbonyl

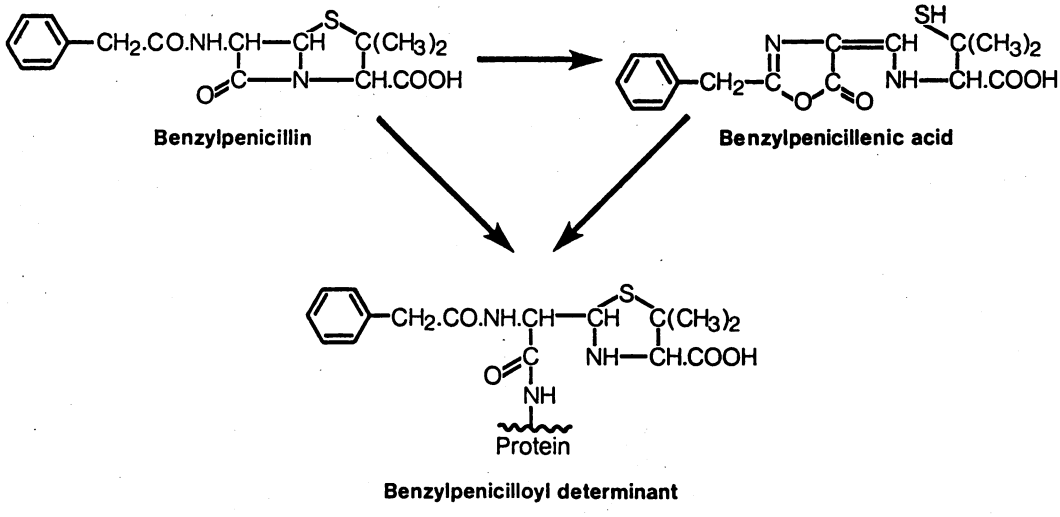

Figure 1. Routes to the formation of the benzyl penicilloyl determinant

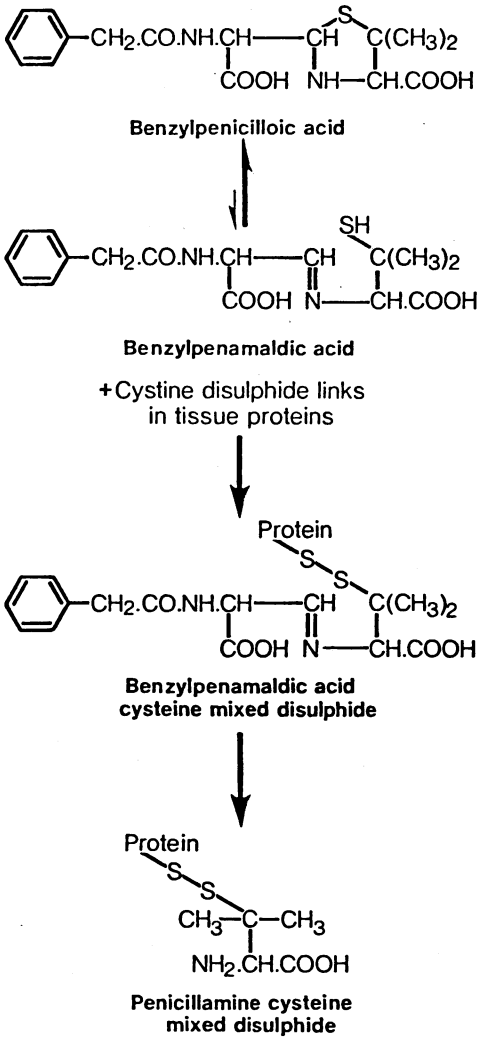

Figure 2. Possible pathway to the formation of a thiol-linked determinant from benzyl penicilloic acid

group. However, there are significant differences and extrapolation is not always possible. An earlier review addresses a number of these immunochemical points (Dewdney 1977).

Within this general framework, routes to the formation of penicillin-related allergens, consequent upon the use of intramammary penicillin preparations to control bovine mastitis, can be defined. Table 2 lists these putative allergens, and draws attention to the fact that they may form *in vivo* following absorption of penicillin or its degradation products from the gastrointestinal tract or they may be preformed and present in ingested milk.

There is no doubt that penicillin and its degradation products can be found in milk

Table 2. Putative penicillin-derived allergens in milk

Preformed or complete allergens	Pro-allergens or haptens
Penicilloylated bovine proteins	Penicillin
Penicillin-derived polymers	Penicillenic acid Penicilloic acid

following therapy for mastitis. There is, therefore, the theoretical risk that sensitization *de novo* or reactions in the already sensitized patient could occur. Quantitative arguments which assess the probability of such events taking place are presented in the next section. Such arguments are based on the related factors of the amount of penicillin likely to be ingested in this form and on the concentration of penicilloylated autologous proteins so formed. As will be discussed, such substituted proteins have been detected in experimental studies (Wal 1980) but only following extremely high intramuscular doses of penicillin, not following ingestion of penicillin in milk.

It is worth drawing attention to further studies by Wal (1980) although they are unlikely to alter the overall quantitative assessment of risk. In these studies it was shown that there may be underestimation of the degree of penicilloylation of autologous protein by radioimmunoassays because some penicilloyl groups may be masked and only exposed on enzymic degradation of the protein. It is conceivable that this could lead to an individual being exposed to penicilloylated proteins over the period of normal protein catabolism *in vivo*, but it remains to be established that protracted penicillin allergic reactions are mediated in this way.

Preformed allergens, in the form of penicilloylated bovine proteins, have been detected in milk obtained shortly after the infusion of high levels of penicillin into the teat canals of the bovine mammary gland. Quantitative aspects of this study (Wal & Bories 1975) are discussed later but it is important to understand that for these penicilloylated bovine proteins to be allergens, they must first be absorbed from the gastrointestinal tract following milk ingestion. No data are available on the absorption of penicilloylated proteins, but studies on food allergy show quite clearly that intact protein can be absorbed through even the healthy gut mucosa. This may be exaggerated in the infant, perhaps due to deficiency of secretory IgA antibody, and in adults consequent upon some inflammatory gut disorder. The major milk protein allergens, α-lactalbumin, β-lactoglobulin and bovine serum albumin, can be absorbed by the human gut under these circumstances and may give rise to cows' milk allergy (Lessof & Buisseret 1981). It can be assumed, therefore, that penicilloylated proteins could be similarly absorbed. Whether low levels of such penicilloylated proteins could be responsible for penicillin allergy is quite unknown but quantitative arguments will be presented shortly.

A further source of preformed allergens to be considered are the dimers, oligomers and polymers (*see* Dewdney 1977). It has been shown that penicillins can polymerize in solution and routes to the formation of polymers, and their structures, have been described. In animals it can be shown that these substances are antigenic but their role in penicillin allergy has not been established. In general, it would seem that at least the larger polymeric forms could be expected to give allergic reactions in penicillin-allergic patients without the necessary requirement of prior protein reactivity. No information is available on the presence of polymeric forms in bovine milk and it is difficult to speculate on this question in the absence of knowledge of penicillin concentrations and the pH of the environment in the udder, both of which would have a significant influence on rates of polymer formation.

Quantitative aspects of primary sensitization and challenge

It will be apparent that it is possible to define, and in some circumstances to measure, potential allergens derived from penicillin in milk supplies. The critical factor to be established now is the possible importance of these allergens from a quantitative aspect. There is no doubt that total penicillin exposure of any individual through milk ingestion is low. Market research figures can be used to attempt to quantify this exposure. For example, in 1980, the average medical use of the penicillin group of antibiotics represented approximately 2.4 g per individual. This contrasts with the figure of 1.3 mg that theoretically could be ingested in milk by an individual in a year, assuming an average yearly consumption of 133 litres of milk and a maximum penicillin level of 0.01 μg/ml. A person drinking 250 ml of milk a day could receive a maximum of 2.5 μg of penicillin; a patient receiving oral therapy would receive at minimum 1–2 g.

The comparative risk from these two sources of penicillin exposure seems obvious, but it is important to assess that risk both from the point of view of primary sensitization and from risk of eliciting reactions in sensitized patients.

Primary sensitization

Preformed allergens: Analysis of the experimental data of Wal & Bories (1975) provides a basis for the quantitation of penicilloylated proteins in cows' milk. In their study, 600 mg of benzylpenicillin[1] was infused into each mammary gland quarter and penicilloyl residues were measured by radioimmunoassay in milk obtained twice daily for four days. The maximum level of penicilloyl residues recorded was in the first milking after treatment and was 1.5 μg/g of milk. Assuming that the penicilloyl residues were covalently bound to bovine albumin, the degree of penicilloylation would thus be very low, at 0.009 groups/protein molecule. This figure is derived by the following calculation. If the assumption is made that milk contains approximately 3.3 g protein/100 ml, then one gram of milk contains 33 mg protein. If the assumption is then made that the protein is albumin of molecular weight 68 000 (the calculation would not be significantly different for the other major milk proteins) then in one gram of milk there are 490 nmol of protein $\left(\frac{0.033}{68\,000}\right)$. The 1 g of milk contained 1.5 μg of penicilloyl residues (Wal & Bories 1975) of molecular weight 335 as free acid, or 4.5 nmol $\left(\frac{0.0000015}{335}\right)$. Since the penicilloyl residues must be bound to protein amino groups, the 490 nmol of protein contain 4.5 nmol of penicilloyl and 1 mol of protein would contain 0.009 mol of penicilloyl. A substitution rate of this order means that one molecule of protein in 111 would contain one penicilloyl group and the remaining molecules would be non-penicilloylated. The number containing more than one penicilloyl group would be exceedingly low and cannot be calculated.

Again, on the assumption that milk contains approximately 3.3 g protein/100 ml, the 1.5 μg penicilloyl residue assayed would be contained in 33 mg protein or in 1 ml milk. Thus overall, 1 ml milk could contain 300 $\left(\frac{33}{111} \times 1000\right)$ μg penicilloylated protein and a person drinking 250 ml of milk could receive, on this calculation, 75 mg of penicilloylated protein.

It might be assumed that this level of protein could represent an immunogenic dose for man were it not for three factors militating against such a role.

The first of these factors is the extremely low level of substitution of the protein carrier by penicilloyl groups. Numerous studies have shown that lightly substituted conjugates are very poorly, if at all, immunogenic with respect to responses specific for the hapten group. Kristofferson *et al.* (1977), for example, found that the epitope density of a variety of penicilloyl protein conjugates had a very significant influence on immunogenicity. Conjugates carrying an average 0.6 penicilloyl residues per protein molecule failed to induce penicilloyl-specific antibody whereas those of higher levels of substitution did so readily. At the calculated substitution level of 0.009 groups per protein molecule arising from penicillin in milk, it seems therefore unlikely that hapten-specific antibody would be generated.

It should be noted that in the experimental studies from which these calculations are derived, an intramammary dose of 600 mg infused into each teat canal was used. This is in excess of normal therapeutic doses of 100–300 mg. It seems probable, therefore, that in clinical practice even these substitution levels would not be achieved.

The second factor which must be taken into account in assessing the immunological hazard of these preformed penicilloyl conjugates in milk is that they are ingested by man. There is very little quantitative data in the literature on the immunogenic dose of a protein or a substituted protein by the oral route. Reference to the literature on food allergy, and in

[1]Dosages and concentrations of penicillins quoted in this paper have been derived by the authors from figures in the original literature in units on the basis of 1.67 units of benzylpenicillin = 1 microgram, or 1.0 units = 1 microgram for the procaine salt.

particular to cows' milk allergy, is helpful in that the syndrome clearly demonstrates the possibility of absorption of proteins through the gut mucosa and the development of an antibody response to them, but quantitative considerations are, of course, totally different.

Some perspective might be gained by consideration of other situations involving low-dose immunization. In the inhalant allergic conditions, for example, pollen hayfever, it is likely that the total inhaled allergen dose in a pollen season is of the order of 100 μg and this is clearly sufficient when repeated seasonally to sensitize patients (Platts-Mills 1981). Moreover, studies in animals have consistently shown that repeated low-dose immunization schedules are the most effective way of producing IgE antibody responses (Levine & Vaz 1970) and it may be that this is true also of man, but in these studies parenteral routes were used.

The third factor relates to the kinetics of the appearance of penicilloylated proteins in milk after mastitis therapy. Using a radioimmunoassay which detects penicilloyl groups, it has been shown that penicilloylated proteins in milk follow a similar elimination curve to that of penicillin itself (Wal & Bories 1975). In this experimental study, when peak concentrations of penicilloyl residues were 1.5 μg/ml, penicillin titres were 22.5 μg/ml. By the eighth, twelve-hourly milking, the penicilloyl residue was almost undetectable at 0.002 μg/ml but the penicillin level remained above the acceptable level of 0.01 μg/ml. Thus a milking-out time appropriate for the penicillin residues would be more than adequate to eliminate the possiblity of penicilloyl residues at significant concentration being present.

Against this background, therefore, it is difficult to sustain the view that the low levels of preformed allergens present in bovine milk after very high-dose treatment represent a significant hazard for man with respect to sensitization capability.

Pro-allergens or haptens: It is even less likely that penicillin *per se,* acting as a pro-allergen or hapten, could be a significant immunogenic stimulus. It is known that the degree of penicilloylation achieved *in vivo* by penicillin therapy is low. In a experimental study by Wal (1980), for example, in which a very high intramuscular dose of benzylpenicillin (6g) was administered to a pig, covalently-bound penicilloyl residues could be detected at a peak level of little more than 10 ng/mg albumin, representing 0.002 penicilloyl groups per protein molecule.

The lack of immunogenicity of such lightly substituted proteins has already been discussed, and in this situation there is a further factor which would minimize the immunogenic challenge of such conjugates. It is known that substituted autologous proteins are significantly less immunogenic than are equally substituted foreign proteins. For example, it has been shown recently that penicilloylated autologous proteins are very poor immunogens even using immunizing schedules designed for maximum response (Ahlstedt *et al.* 1979). Bearing in mind the very low oral dose that could arise from penicillin at the accepted level in milk, that is some 2.5 μg in a 250 ml drink, and the consequently minimal levels of penicilloylated autologous proteins that could be generated, the risk of sensitization can essentially be ruled out.

Reactions in sensitized individuals

Preformed allergens: Much the same considerations apply to the role of penicilloylated proteins in eliciting reactions in sensitized individuals. The extremely low level of hapten substitution likely to be generated means that the number of even monosubstituted proteins is low and of multisubstituted, orders of magnitude lower. A minimum of disubstitution is a general requirement for a challenging antigen (Levine 1965, Kristofferson *et al.* 1977) and, in these circumstances, is not achieved.

Pro-allergens or haptens: Regarding penicillin itself, it is known that oral therapy can result in generalized allergic and anaphylactic reactions in penicillin-allergic patients but the incidence of such reactions is relatively low. Idsøe *et al.* (1968) in an analysis of fatal anaphylactic reactions, found that only 2% were elicited by oral therapy. A more recent

review of world literature records only 7 cases of serious reactions after oral penicillins (Becker 1976). It is impossible to judge from these cases the minimum dose of penicillin which would have elicited reactions in these patients, as all had received full therapeutic doses within the range of 0.25 to 1 g.

There are several clinical reports in the literature of low doses of penicillin used in skin tests or following inhalation eliciting systemic reactions (Bierlein 1956, Reisman & Arbesman 1968) but clearly the routes of administration are inappropriate to an analysis of oral challenge doses. There are, however, some reports which can be used to address this question. The best evidence is provided by a patient investigated by Borrie & Barrett (1961). Severe allergic reactions developed during an attempt at oral desensitization with penicillin within a dose range of 3–9 μg. Wicher *et al.* (1969) and Wicher & Reisman (1980) reported a well investigated patient who responded systematically to penicillin in milk and in a soft drink. The eliciting doses were estimated to be of the order of 1359 μg in the milk and 16–34 μg in the soft drink. In a case recorded by Vickers *et al.* (1958) the eliciting dose may have been of the order of 2400 μg. The difficulty in this type of analysis is that the minimum challenge cannot be assessed and, as the assays for penicillin were done some days after the event, actual penicillin levels may have been higher than the assay figures suggest. However, it is possible to get some insight into minimal doses from analyses of this kind.

Tscheuschner (1972) estimated that less than 6 μg of penicillin must have been responsible for eliciting a reaction in a patient who had an allergic reaction while eating pork contaminated with penicillin, although in 9 well-documented penicillin-allergic patients investigated by Lindemayr *et al.* (1981) 3.6–6 μg penicillin were tolerated when ingested in raw pork. Clearly there are limits to the information which can be obtained from data of this kind and direct challenge experiments (Lindemayr *et al.* 1981) have inherent dangers. To overcome this problem Siegel (1959) used the Prausnitz-Küstner (PK) reaction in which serum from allergic patients is injected intradermally into the skin of normal recipients. After a latent period the recipient is given the allergen, in this case penicillin, by an appropriate route. Siegel (1959) investigated, by this technique, a number of sera taken from different patients. Serum from one patient responded to an oral penicillin dose of 30 μg with a clear positive response including whealing, and 24 μg was sufficient to give an erythematous reaction. Assuming that the sensitized patient is, at minimum, 100 times more responsive than the recipient in a PK reaction, Siegel then argued that the threshold dose to challenge a sensitized person would be 0.24 μg. This figure, 0.24 μg or 0.4 unit, has been widely quoted in the literature. It is important, therefore, to understand that it is an extrapolated figure and one significantly lower than those calculated from patients responding to penicillin ingestion. This is particularly relevant to the question of penicillin residues in milk. An individual drinking 250 ml of milk containing the accepted level of 0.01 μg/ml could ingest 2.5 μg penicillin, a low figure relative to most of the data on challenge doses.

However, it is clear from the data available that penicillin-allergic patients could respond to low levels of penicillin by ingestion, although they do so very rarely if the published literature is an adequate guide. The next section of this paper assesses the clinical evidence that adverse reactions in allergic patients have been induced by such low-dose exposures to penicillin in milk.

Clinical evidence

As long ago as 1948, it was known that milk from penicillin-treated cows could inhibit cheese-starter cultures. Over the next decade, understanding of the mechanisms involved in the development of penicillin allergy increased and it was not unreasonable that the public health significance of the presence of penicillin allergens in milk should be questioned. Milk and milk products were regarded as but one source of covert exposure to penicillin and attention was drawn to residual penicillin in sterilized syringes and from penicillin-producing fungi in the environment (Coleman & Siegel 1955, Siegel 1959). Welch (1957) expressed this concern in a publication which recorded that the Commissioners of the Food and Drugs

Administration of the USA had authorized the setting up of a Medical Advisory Panel to consider public health problems arising from the presence of antibiotics in marketed milk. The Panel took the view that of the antibiotic residues that might be present in milk, only the case of penicillin required consideration. However, Welch (1957) stated at the time that 'We do not have a single proved case of a reaction following the ingestion of fluid milk known to contain penicillin', and he emphasized that the problem of contamination of milk with antibiotics was a small one compared with other food safety problems, including those created by the use of preservatives, colouring agents, stabilizers and flavours.

Eighteen years later, Olson & Sanders (1975), also of the FDA, wrote: 'The problem appears to be of such magnitude that would seem to require the co-ordinated effort of all producer-cooperative organizations to bring the problem under control – and the sooner the better for all concerned'.

The literature has been searched diligently to find the evidence which would support such a dramatic change of view. Two types of hazard or clinical situation were sought. In the first, the published literature was reviewed to establish whether any case histories presented could be interpreted as supportive of the view that residual penicillin in milk could induce sensitization in man *de novo*. In the second, the question was asked whether there were documented cases of the ability of residual penicillin in milk to elicit reactions of an allergic nature in patients with a clinical history of penicillin allergy.

The first is readily answered. There are a few anecdotal cases quoted in the literature (Mauranges 1972, Cany 1977) but none is well-documented and in spite of the theoretical risk of ingesting low concentrations of an allergen over an extended period, referred to earlier, the consensus of those with an interest in this topic is that primary sensitization is a negligible risk.

Before analysis of the second question, that of allergic reactions in penicillin-allergic individuals, it is important to establish the criteria on which the evidence is based. Table 3 lists criteria in an order which gives increasing confidence in the accuracy of the diagnosis. Table 4 summarizes the clinical cases documented in the literature, and notes the diagnostic criteria used in each case.

Several points may be made in discussion of these findings. It is clear that there are very few documented cases, a conclusion reached also by Becker (1976) and Adkinson (1980). It could be argued that clinical reactions do occur due to penicillin residues in milk, but either these are misdiagnosed or individuals, recognizing that their symptoms are associated with ingestion of milk, act on their own initiative and simply avoid milk and milk products. A recent survey (Harris 1982) is relevant to this issue. It was shown that, of a survey sample of 8749 people in the UK, 1.5% avoided milk because they perceived it to do them some harm. This group would be expected to include those with cows' milk allergy, lactose intolerance and other syndromes. It is possible that the group also includes some who are allergic to penicillin residues in milk and although their perception of the precipitating cause of their symptoms is correct, the precise aetiology is not apparent to them. However, in view of the low incidence of milk avoidance in the population, based on this survey, and the probable dominance of cows' milk allergy and intolerance in determining avoidance, it is unlikely that there exists in the UK a significant percentage of individuals whose penicillin allergy precipitated by penicillins in milk has gone undiagnosed.

Analysis of data given in Table 4 shows that even the cases that are recorded cannot be

Table 3. Diagnostic criteria used in investigations of penicillin allergy induced by milk

A	Clinical history of adverse reaction to milk ingestion in penicillin-allergic patient
B	Clinical evidence of improvement in condition on milk avoidance
C	Clinical evidence that milk known to be free of penicillin, or penicillinase-treated milk, can be tolerated
D	Demonstration of penicillin residues in milk sample which precipitated adverse reaction
E	Clinical evidence of the prophylactic or therapeutic value of penicillinase
F	Immunological evidence to support diagnosis of penicillin allergy and/or lack of sensitivity to milk proteins

Table 4. Summary of reported cases of penicillin-specific allergic reactions elicited by milk

Reference	No. of cases	Clinical response	Criteria (see Table 3)	Comments
Erskine 1958, in Mauranges 1972	1	Dermatitis	A,D	No further details available. Original reference not examined
Vickers *et al.* 1958	2	(1) Contact dermatitis	A,C	Positive patch test to penicillin. No clinical history. No evidence of penicillin in offending milk sample. Intramuscular desensitization claimed to be successful
		(1) Rash, lymph-node enlargement; joint swelling	None	Positive patch test to penicillin but no clinical history of penicillin allergy. Circumstantial evidence only
Zimmerman 1957–8, 1959	4	Chronic urticaria, hives	A,C,E	Importance of dairy products, Roquefort cheese stressed. See text for interpretation of criterion E. No evidence (other than indirect through action of penicillinase) of penicillin in these products
Siegel 1959	1	Generalized immediate hypersensitivity	A,F	A highly sensitive penicillin allergic through IgE mechanisms but evidence for involvement of milk anecdotal. Criteria C and D not fulfilled
Borrie & Barratt 1961	1	Dermatitis	A,B,C,D,F	Best documented case but diagnosis only confirmed by challenge tests. PK test performed but negative
Smythe 1969	1	Dermatitis, angio-oedema. Recurrence of undulant fever	B	No evidence given of basis of diagnosis of penicillin allergy. No evidence of penicillin in milk consumed
Wicher *et al.* 1969	1	Pruritis, rash, headache	A,D,F	Well-documented penicillin allergic. Immunology and skin tests indicate IgE-mediated reaction
Cany 1977	3	Urticaria	A,B,F	Patients said to be sensitive also to pork, veal, chicken. No evidence that penicillin present in offending milk or meat

said to provide unequivocal evidence of the hazard of penicillin in milk. The diagnostic criteria used in most cases are inadequate in one respect or another. In very few cases was the presence of penicillin shown in the offending milk sample and only rarely was there sufficient diagnostic evidence of either penicillin allergy or of lack of milk intolerance.

The use of penicillinase is of interest. Penicillinases (β-lactamases) are enzymes which hydrolyze penicillins to penicilloic acids by opening the β-lactam ring. A penicillinase (Neutrapen) was first used in this context by Becker (1956) and evaluated extensively by Zimmerman (1957–1958). It is no longer used in most countries of the world as therapy in penicillin allergy partly because of dangers of sensitization and partly because current immunological theory indicates that breakdown products of penicillins might play a role in eliciting penicillin allergy. If penicillinase therapy were responsible, in the cases listed, for the benefit obtained in allergic patients treated with it, it would be justifiable to conclude, first that penicilloic acid and its further degradation products (see Figure 2) play no part in penicillin allergy induced by penicillin in milk and, furthermore, that penicilloylated bovine protein conjugates which are unaffected by penicillinase are equally blameless.

Overall, therefore, although it is established that penicillin in milk can elicit reactions in some individuals, the clinical and scientific validity of many of the case reports needs to be evaluated critically in the light of current knowledge and techniques. There seems little in the literature to support the view that there is a problem of significant magnitude and the dramatic view expressed by Olson & Sanders in 1975 seems unwarranted.

Boonk & Van Ketel (1980, 1982) have reported a study which may have some indirect bearing on the question of the significance of penicillin in milk. In an investigation of 252 patients with chronic, recurrent urticaria, 70 (27.8%) showed either patch test reactions or intradermal responses to penicillin derivatives. Thirty out of 52 of these patients responded clinically to a diet free of milk or milk products, whereas only 2 out of a group of 40 patients with chronic urticaria and negative skin tests responded favourably to this diet restriction. This study is of interest but cannot be used to support the view that penicillins in milk are of clinical significance. No evidence was presented that there was any penicillin in the milk consumed by these responding patients and sensitivity to milk proteins was not ruled out. The prevalence of positive skin test reactions to penicillin was remarkable, and the significance of the results must be questioned in the context of the fact that in only 7 patients out of 99 questioned was there any evidence of a clinical history of penicillin allergy. It is possible that mast cell fragility might have been responsible for this high frequency of skin test reactions in this particular group of patients. Chronic urticaria is a heterogeneous condition of mixed aetiology and the severity of the disease is subject to fluctuations. Firm conclusions cannot yet be drawn on these data, but the role of penicillin allergy in chronic urticaria bears further investigation whether related to milk as a source of penicillin allergens or not. Penicillin in therapeutic use is possibly the commonest cause of drug-induced acute urticaria, but no evidence is available on which to base a mechanism of action for any role penicillin may have in chronic urticaria. Hidden sources of penicillin are usually blamed but no evidence has been presented (Jillson & Porter 1965, Warin & Smith 1976).

The data of Zimmerman (1957–1958) on the use of penicillinase in the treatment of penicillin-allergic patients with chronic urticaria suggest that the presence of the penicillin molecule with an intact β-lactam ring is necessary for the prolongation of the urticarial lesion. Thus when penicillinase was used in therapy the episodes of urticaria were aborted. It might be concluded that this implies that if chronic urticaria can sometimes be due to covert penicillin in milk, then either one has to postulate continuous or at least frequent intermittent exposure to intact penicillin or consider an alternative source of persistent allergen.

In a different context, that is following systemic treatment of pigs with benzylpenicillin, Wal (1980) has shown that penicilloylation of porcine serum albumin can occur and that these modified albumins are eliminated by two routes. Rapid elimination follows the interaction of conjugate with antibody to the penicilloyl group, but Wal claims that some penicilloyl groups may be masked inside the tertiary structure of the protein and are thus unavailable to antibody interaction. Normal catalytic breakdown of these proteins would, however, unmask these groups, exposing them to antibody and giving rise to a persistent allergen which could account for late and chronic allergic reactions. Further investigation of this phenomenon is warranted. However, it does not explain the apparent efficacy of penicillinase in the treatment of penicillin allergy elicited by milk, as this enzyme has no effect on penicilloylated proteins.

The work also opens to question the site of penicilloylation of serum albumin, and this requires further study as it has implications beyond the narrow issue of penicillin residues in milk.

Discussion

It has proved difficult to quantify the public health significance of penicillin residues in milk. Clarification of the mechanisms involved in penicillin allergy has led to it being regarded as the model system against which other drug-induced allergic syndromes are measured. There are advantages and disadvantages in this honoured position. Research on the nature of penicillin allergens, including polymers and macromolecular impurities, has led to more attention being paid to the purity and stability of penicillins and this, coupled with the greater use of oral penicillins, has resulted in a lower incidence of clinical reactions. On the other hand, because the propensity of penicillin to cause allergic reactions is so well known, there is a tendency to over-diagnose, and an adverse reaction of an allergic type in a

patient receiving a penicillin will thus, on probability grounds alone, be diagnosed as penicillin allergy without due attention being paid to alternative diagnoses such as pseudo-allergic reactions.

Similar considerations apply in relation to penicillin in milk. There is no doubt that mechanisms exist whereby penicillin-derived allergens might be both immunogenic and allergenic and the nature of the allergens can be, at least in part, defined. There is equally no doubt that a few individuals are so hypersensitive to the penicillin group of antibiotics that they respond adversely to very low challenge doses administered by a variety of routes. These data, however, lead only to the conclusion that penicillins in milk could represent an important covert source of penicillin allergens hazardous to allergic people. From the literature reviewed in this paper, it is clear that this must be regarded as a possibility. On the other hand, the probability that, at currently accepted levels, penicillin residues represent a hazard, is low. Clinical evidence supports this view in that in spite of intense interest in the topic over many years and costly regulatory involvement, only a very small number of individuals are documented as having had allergic difficulties.

The question that must now be addressed is whether it is justified, in response to ever-increasing assay sensitivity, to aim to reduce still further penicillin residues in milk. This course of action seems unjustified, a view expressed also by Becker (1976). It may be that there will always be one or two individuals of such exquisite sensitivity that they will respond to submicrogram concentrations of penicillin. Public health regulations, however, cannot be expected to accommodate such individuals who will learn to avoid foodstuffs which seem to do them harm in the same way that food-allergic subjects do. The cost-benefit equation should dominate public health concerns and no immunological benefits are likely to accrue from further lowering of acceptable levels of penicillin in milk.

Moreover, there would seem to be no justification in introducing an assay for the presence of penicilloylated proteins in milk. The evidence presented in this paper fails to establish a significant role for them in penicillin allergy and the kinetics of their disappearance from milk parallels that of penicillin itself. In the experimental work quoted (Wal & Bories 1975), such penicilloylated proteins had reached undetectable levels at a time after therapy at which there was still 0.06 ppm penicillin, a level above that now acceptable. The simpler assay for penicillin in milk, compared with radioimmunoassay for penicilloylated proteins, would seem therefore to offer an adequate safeguard of the quality of milk.

Finally, this paper has been addressed to the question posed in its title. The significance of the presence of other antibiotics which might be found in milk as a consequence of treatment of bovine mastitis has not been considered. It is probable that most of the comments made apply equally to the cephalosporins, which, as β-lactam antibiotics, are capable of reactivity similar to that of the penicillins.

The role of tetracyclines and aminoglycosides in inducing allergic reactions has been reviewed (Dewdney 1977). These antibiotics can participate in immunologically-mediated reactions but it is unlikely that their presence in milk could, as a consequence, be a clinical hazard.

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